

A Determination of the Effects of Nitrogen and
Terpenes on Spruce Budworm Growth and Survival

Study File

A DETERMINATION OF THE EFFECTS OF NITROGEN AND TERPENES
ON SPRUCE BUDWORM GROWTH AND SURVIVAL

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SUMMARY

Studies on the resistance - susceptibility characteristics of hosts to the western spruce budworm have been, until recently, generally lacking. Recent studies have shown that variation in foliage quality characteristics in natural populations of Douglas-fir trees has a significant effect on levels of budworm success. The objective of this research was to determine the effects of varying levels of nitrogen, bornyl acetate, and beta-pinene on budworm success. Three different populations of budworm were used in the research. Overall the New Mexico population grew more slowly than the population from Idaho, but the difference in growth rate was significantly less under low nitrogen conditions. The growth response to beta-pinene was different depending on the level of beta-pinene in the diet, but in general beta-pinene seems to be a feeding stimulant. High concentrations of bornyl acetate always depressed growth rates but the effect was somewhat reduced under low nitrogen conditions. The frequency of survival to adult stage was 3 times greater when bornyl acetate concentration was low than when the concentration was high (20.5% vs 7.1%). The data from this study indicate that the general patterns of reduced success due to defensive chemistry is verified for some of the constituents.

INTRODUCTION

Spruce budworm - host associations have been studied extensively for several years and considerable progress has been made toward understanding some of the major factors and processes affecting budworms (Morris, 1963; Johnson and Denton, 1975). Included are the relationship between climate and the population dynamics of budworms, budworm dispersal, the number of and preference for various host species, defoliation, the importance of silvicultural regimes, and the effect of insecticides as well as predators, parasites, and pathogens on budworm populations. Much is still to be learned about most of the above, but obviously missing items in this list are those dealing with the effect of host foliage quality on budworm populations, such as the (1) resistance - susceptibility characteristics among and within host species, and (2) an investigation of the ways foliage quality aspects affect budworm growth and fecundity.

Some of the major characteristics determining the suitability of foliage to phytophagous insects are soluble protein and carbohydrate concentrations and natural chemical defenses of hosts (Rhoades and Cates, 1976; Mattson, 1980; McNeil and Southwood, 1978). Numerous articles have been published demonstrating the

adverse effects of these chemicals on phytophagous insect feeding behavior, larval growth, and adult fecundity (Rosenthal and Janzen, 1979; Rhoades and Cates, 1976; Hanover, 1975; Mattson and Addy, 1975).

Examples do exist indicating that chemical defenses can confer resistance to budworm. Heron (1965) in laboratory bioassays found that the glycoside pungenin has a deterrent effect on eastern spruce budworm larval feeding. Wilkinson (1970) discovered a compound similar to pungenin which constitutes a major chemical difference between the more budworm resistant black spruce and the susceptible red spruce. Species hybrids have varying levels of both pungenin and resistance to eastern spruce budworm attack although statistical analysis of the data are lacking. Mattson and coworkers have found from laboratory studies that various terpenes and tannin extracts from hosts adversely affect the eastern budworm (Mattson and coworkers, CANUSA Newsletter, 1983).

For the western spruce budworm, studies on the resistance - susceptibility characteristics of hosts, until recently, were generally lacking (Johnson and Denton, 1975). Recent studies by Cates and coworkers (Cates et al. 1983a, 1983b; Redak and Cates, in press) have demonstrated that variation in foliage quality

characteristics can have significant effects on budworm success. In a study using a natural budworm population in Montana, 50% of the variation in budworm infestation intensity (density x defoliation levels) was explained by 9 variables, 6 of which dealt with terpenes and nitrogen. With regard to this report, the acetate fraction was inversely related to infestation intensity while β -pinene was related positively to levels of density and defoliation. In a study conducted in New Mexico, 35% of the variation in female dry weight production was explained by 11 variables, 7 of which dealt with terpenes and nitrogen content of the foliage. In another study conducted in New Mexico, Douglas-fir trees growing on south facing slopes were water stressed by trenching. The response of budworm to the foliage quality of these trees was compared to that of budworm reared on the foliage of trees growing on a north facing slope (control). Budworm larvae survived at a greater level (69%) and females were 30% heavier when reared on stressed trees compared to those reared on the non-stressed, control trees. Associated with the foliage of stressed trees were significantly higher levels of alpha-pinene and soluble nitrogen while the foliage of the non-stressed trees was lower in the above 2 foliage parameters but higher in bornyl acetate, beta-pinene, and 2 unidentified terpenes. It is interesting to note that in no case were tannins important in determining larval or adult success.

In each case the acetate fraction of the foliage extracts, but particularly bornyl acetate, beta-pinene, and nitrogen content, was implicated in influencing budworm success. As a result these parameters were used in the synthetic diet study to determine if any cause and effect relationship between these foliage quality variables and budworm growth and survival could be determined.

OBJECTIVES

The concept of tree resistance has been defined by Cates and Alexander (1982), Hanover (1975), and Beck (1965) as "the collective heritable characteristics by which a plant species, race, clone, or individual may reduce the probability of successful utilization of that plant as a host by an insect species, race, biotype, or individual." We are interested, then, in the foliage quality characteristics that have an adverse influence on the population dynamics of WSB at the larval and adult levels.

The objective of this research is to determine the effect of various foliage quality characteristics on larval growth and adult fecundity in the laboratory using a synthetic diet. This will be accomplished by investigating the following questions:

1. Do budworm larvae from 3 populations reared on synthetic diets containing varying amounts of nitrogen, beta-pinene and bornyl acetate show reduced larval growth and adult survival?
2. What effects do the interactions between nitrogen, beta-pinene, and bornyl acetate have on budworm larval growth, survival, and adult survival?

METHODS

Initial preliminary experiments used budworm larvae obtained from the laboratory colony maintained by Dr. J. Robertson, Pacific SW Forest and Range Experiment Station, Berkeley, California. In addition, the experiments reported here involved budworm larvae obtained from natural populations located near Mac's Inn, Idaho and Barley Canyon in the Jemez Mountains near Jemez Springs, New Mexico. Budworm were obtained from all populations as pupae. Pupae were maintained until emergence and adults were allowed to mate and lay eggs in paper bags that contained a sprig of Douglas-fir. No interbreeding among populations was allowed. Egg masses were disinfected and newly hatched larvae were reared in a controlled environmental chamber on standard CANUSA agar diet (Robertson, 1979). Larvae were reared to the third instar, at which time they were placed on experimental diets.

Newly molted, third instar larvae were selected from each population and were assigned arbitrarily to be reared on one of 8 diets. The design for the experimental diets included three factors: total nitrogen, beta-pinene, and bornyl acetate all of which were present in low or high concentrations. For our experiment, levels of each of these factors were chosen to approximate the naturally occurring extremes (Table 1). Previous studies of primary

and secondary constituents of Douglas-fir needle tissue from Montana and New Mexico provided the initial data on the natural high and low levels (Cates et al., 1983a). The CANUSA diet was modified to simulate the high and low levels by adjusting the proportion of caesin and wheat germ in the diet. The terpenes used were purified standards obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin 53233, U.S.A.

To obtain the desired nitrogen levels for the 8 diets, caesin and wheat germ contents of the diets were manipulated based on calculations of the amount of nitrogen in each source. The diet was then made up and 3 samples, randomly selected, were analyzed for total nitrogen. After this procedure, refinements needed to obtain the exact level were carried out by again varying the 2 nitrogen sources in the diet and determining the nitrogen content. These levels were then maintained each time the diet was made.

Because of the high volatility of the terpenes, higher amounts than needed were added and blended thoroughly in the hot, liquid diet. Also, because the 2 terpenes varied in their degree of volatility, this procedure had to be carried out separately for each terpene. The various degrees of volatility for the 2 terpenes at the temperature of the agar diet when the terpenes were added, were calculated, and a larger amount than needed was added to the diet.

After the agar diet cooled, 3 randomly selected samples from each of the 8 diets were analyzed on the gas chromatograph using the conditions outlined in Cates et al. (1983b). Refinements in the amounts added were made as needed until the conditions for the terpenes in Table 1 were repeatable. During the experiment, 3 samples, randomly taken from every diet, were run periodically on the gas chromatograph to insure that the terpene content did not vary significantly from batch to batch, and to insure that each diet was different consistently at the same level. In addition, the diets were changed every other day to further reduce differential volatility problems.

Larvae were individually caged in sterile polystyrene petri dishes (6 cm dia.). Approximately 5 g of experimental diet was included in each dish and was replaced every other day. A small disk of filter paper was placed in each dish to absorb condensation and prevent drowning of the larva. Petri dishes were randomly distributed among shelves within the environmental chamber and status of each larva was checked daily.

With the exception of the larvae from the laboratory colony, all larvae were weighed at the outset of the experiment. Terminal weight was determined immediately upon death of the larvae or at pupation. Initial pupal weight is not an entirely accurate measure of terminal larval weight due

to high metabolic costs of pupation. However, it was felt that the disturbance of pre-pupation larvae was not desirable (Reese, 1983). Secondly, the estimation of the exact onset of pupation from individual larvae is difficult and may introduce as much error in the final larvae weight as simply weighing newly formed pupae. Lastly, all pupae that formed were maintained until death or adult emergence, and the number of adults produced per diet was recorded.

RESULTS

Per day growth rate was calculated for Idaho and New Mexico populations by dividing total weight gain (or loss) by the number of days the larva survived or remained until pupation. The range of daily growth increment (DGI) that resulted for both populations was from -3.73 mg/d to +564 mg/d. Data were normalized by computing the square root of $DGI + 3.733$. The value 3.733 was merely a scaling factor to avoid problems associated with taking the square root of a negative number. The transformed data were then subjected to analysis of variance for the effects of population, and levels of nitrogen, beta-pinene, and bornyl acetate. The results showed a significant effect of all four factors (Table 2) and interactions between the effects of nitrogen and each of the other three factors (Figure 1).

Overall the New Mexico population grew more slowly than the Idaho population, however, the differences in growth rate were significantly less distinct under low nitrogen conditions. The effect of beta-pinene was mixed. At high nitrogen concentrations growth was enhanced by high beta-pinene concentrations, but at low nitrogen concentrations the response to high beta-pinene concentrations was nearly identical to that of low beta-pinene concentrations. High concentrations of bornyl acetate always depressed growth rate, but like population differences the effect was some-

what reduced under low nitrogen conditions. Furthermore, daily growth increment at high nitrogen levels accompanied by high bornyl acetate concentrations was depressed nearly as much as when nitrogen was low and bornyl acetate was reduced.

Overall survivorship to the adult stage was low being only 15.8% of the larvae starting the experiment that completed development. Numbers of larvae surviving to adult stage relative to those that died as larvae or pupae were subjected to categorical analysis to determine whether they were distributed independently among populations and levels of nitrogen, beta-pinene, and bornyl acetate. Natural logarithms of counts were used and results showed that differences in the proportion of adults produced existed among populations ($X^2 = 11.4$, $p = .003$), and levels of nitrogen ($X^2 = 33.5$, $p < .001$) and bornyl acetate ($X^2 = 5.2$, $p = .023$). Beta-pinene had no effect ($X^2 = 0.1$, $p > .75$) and there were no interactions among factors. Table 2 indicates counts for adult and larval/pupal categories for the significant factors. The frequency of survival to adult stage was highest for the laboratory population (22.4%) followed by the Idaho population (12.9%) and then the New Mexico population (6.6%). Pairwise comparisons showed that the proportion of adults in the laboratory population was higher significantly than either of the natural populations which did not differ.

Nearly 20 times more adults were produced at higher nitrogen

levels (28.4% vs 1.4%). Abundances of primary nutrients have often been implicated as important predictors of herbivore success (McNeil and Southwood, 1978) and this result verifies that conclusion. While nitrogen in particular has been shown to be toxic at very high levels (Brewer et al., 1980), the response we observed in this study to nitrogen concentrations near the maximum that budworm encounter in nature suggests that nitrogen toxicity is not important in most cases. The response to bornyl acetate, while not as great was still dramatic. The frequency of survival to adult stage was 3 times greater when bornyl acetate concentration was low than when the concentration was high (20.5 vs 7.1%). Interestingly, survival to adulthood was nearly as great when bornyl acetate concentrations were low as when nutritional quality (nitrogen concentration) was high (Figure 1).

CONCLUSIONS

Several conclusions may be drawn from the results of this study, but perhaps the most important is that the secondary compounds present in plant tissues may have important effects on the growth and survival of herbivorous insects, and in this particular case, on the budworm. While the supposition that these compounds have active roles in the defense of plant tissues is now generally accepted (Rhoades and Cates, 1976) in place of older arguments that they are non-functional metabolic end products or waste products, most of the evidence is correlative. We have presented conclusive evidence that a high bornyl acetate concentration strongly depresses growth rate and the numbers of adults produced from a generation of budworm larvae. The depression is such that under otherwise highly nutritious conditions, the result is the same as if primary nutrition (nitrogen or protein in this instance) was deficient. The implications are profound. Larval consumption of plant tissue does not increase linearly as insects develop, but instead is incremental in a multiplicative fashion. Late instars ingest much more leaf tissue per unit mass than earlier instars. Consequently, any mechanism that can impair growth and development, if even in a small amount, results in large benefits in terms of tissue saved from destruction or damage.

Secondly, any prolonging of the normal life cycle increases the chance that the developing insect will succumb to predation or lethal abiotic conditions that may arise during the budworm life cycle, but particularly during the larval stages. While impairment of growth rate has short-term benefits, the reduction of numbers of adult insects produced has obvious long-term effects on the intensity of herbivory to which the host plant will be subjected.

The data dealing with beta-pinene suggest that managers may not only wish to select for resistant trees, but also to remove those that may enhance attraction of budworm or that may stimulate feeding in some way. In our experiemnts, budworm larvae performed better when beta-pinene concentrations were higher, indicating that this compound may indeed stimulate feeding or enhance the efficiency with which larvae assimilate what they ingest (Harborne, 1982).

We have found considerable geographical variation in defensive chemistry of Douglas-fir (Cates et al., 1983a; 1983b; unpublished). The data presented from this study suggest a strong and significant geographical response in the natural budworm populations to varying nitrogen levels. Increasing nitrogen levels will always have an effect, but the level of response was significantly greater in the northern population (Mac's Inn population). Any increase in the nitrogen content of foliage, for example

due to fertilization or drought stress, may enhance the survival, growth, and fecundity of budworm. Furthermore, based on these data, we would predict that the response would be much greater in the northern populations. This suggests that budworm populations in the north may increase quickly due to the disturbance of the natural imbalance between primary nutrition (nitrogen in this case) and defensive chemistry in the foliage as compared to the New Mexico population. This is an important area that we are currently pursuing.

We have shown from both field and laboratory studies that foliage quality is an extremely important variable in budworm dynamics. Based on the data in this report and our other investigations along with Kirkbride and Wagner (1983), silviculturalists may wish to incorporate some of this information into various management regimes in an effort to reduce the adverse affect of the budworm but also other forest pests. Table 4 suggests some ways in which this might be done.

COOPERATION

We are grateful for Jackie Robertson, Pacific Southwest Forest and Range Experiment Station, Berkeley, California, for providing several batches of budworm pupae from her colony while we were conducting preliminary experiments to the one reported here. We are grateful also for her advice and patience during this endeavor which, in the end, was successful!

PROBLEMS ENCOUNTERED

1. Preliminary experiments also showed the effect of bornyl acetate (Cates et al., 1983b). However, those data were not as conclusive as the results of the experiments reported in this paper. The main reason for this is that experimental larvae were not held placed individually in petri dishes. One attempting such a study as this should be certain that larvae are separate so that cannibalism is circumvented. We suspect that it is this supplemental, undefended source of nitrogen that reduced the effectiveness of secondary chemistry in those preliminary experiments.

2. Starting the laboratory colony from Dr. Robertson was difficult, but eventually was accomplished. The directions in Robertson (1979) need to be carefully followed, and with minor modifications based on the type of environmental chamber or environmental conditions that are being used, a colony can be established.

3. Adjusting the CANUSA diet so that it is somewhat near

the foliage quality levels that we wished to mimic is difficult. Much trial and error is involved, and the diet must be monitored carefully throughout the experiment.

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Table 1. Concentrations of experimental components in young needle tissue of Douglas-fir in Montana and New Mexico, and in the artificial diets on which experiments were conducted.

	<u>Low Concentration</u>		<u>High Concentration</u>	
	Natural	Artific. Diet	Natural	Artific. Diet
Total nitrogen (%)	0.75	0.68	2.5	2.27
Bornyl Acetate (mg/g fr. wt.)	201	191	1408	1315
Beta-Pinene (mg/g fr. wt.)	50	68	762	842

Table 2. Daily growth increment for budworm larvae from different populations and at different concentrations of total nitrogen, bornyl acetate, and beta-pinene.

Effect	Daily Growth Increment	
	\bar{x} (mg/day)	N
Population		
New Mexico ^a	-0.197	285
Idaho ^b	0.497	250
Nitrogen		
0.7% ^a	-0.355	292
2.3% ^b	0.707	243
Bornyl Acetate		
191 mg/g ^a	0.459	263
1315 mg/g ^b	-0.193	272
Beta-Pinene		
68 mg/g ^a	0.041	283
842 mg/g ^b	0.224	252

Within each comparison values followed by different superscripts are significantly different at $p < 0.05$

Table 3. Numbers of budworm that survived to adult stage or died as pupae or larvae, among populations and at varying concentrations of nitrogen, bornyl acetate, and beta-pinene.

Category	Adult	Pupa/Larva
Population		
Laboratory ^a	57	197
Idaho ^b	33	223
New Mexico ^b	19	268
Nitrogen		
0.7% ^a	6	428
2.3% ^b	103	260
Bornyl Acetate		
191 mg/g ^a	80	311
1315 mg/g ^b	29	377
Beta-Pinene		
68 mg/g ^a	42	374
842 mg/g ^a	67	314

Different superscripts indicate that proportions differed at $p < 0.05$

Table 4. Recommendations concerning the way in which plant resistance could be incorporated with various forest management practices.

Thinning

- Select for the more resistant chemical phenotypes
- Reduce the proportion of susceptible chemical phenotypes in the stand
- Remove infested, stressed, damaged, and older trees
- Improve individual tree vigor
- Maintain heterogeneity in tree phenology within a stand

Replanting

- With resistant chemical phenotypes normally present in the population
- With resistant chemical phenotypes from other populations
- With varying mixtures and densities of hosts and non-hosts

Fire-Clearcutting

- Maintains diversity in tree age among stands
 - Maintains greater vigor among stands
 - Maintains chemical diversity among hosts
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From Cates et al., 1983b.

FIGURE 1. INTERACTION EFFECTS BETWEEN NITROGEN AND POPULATIONS, BETA-PINENE, AND BORNYL ACETATE

